

Potential dose-adjustment parameters during 6MP/MTX-therapy for Acute Lymphoblastic Leukemia

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Abstract

Acute lymphoblastic leukemia is a clonal acute malignant blood disease that is treated with a combinational therapy involving daily oral 6-mercaptopurine (6MP) and weekly Methotrexate (MTX) until 2-3 years from the time of diagnosis. Proper dosing of 6MP is still a major clinical challenge as the drug exhibits a low therapeutic index and substantial variations among patients. Over- and under treatment remains a great clinical challenge as appropriate dosing is hampered by the complex pharmacogenomics and kinetic characteristics that is unique to each patient. Enzymes involved in the 6MP/MTX metabolism such as TPMT (*Thiopurine S-methyltransferase*) have been screened for polymorphisms and it has been established that TPMT variants are an important genetic cause of inter-individual variability in the clinical response to 6MP. However the inter-individual differences in treatment response cannot be fully explained by TPMT gene variants. Additional genes must be involved in the determination of adverse events to multi-agent chemotherapy. ITPA (*Inosine triphosphate pyrophosphatase*) is also regarded as a great determinant of mercaptopurine metabolism and the risk of toxicity.

Recent studies have evaluated the potential of DNA-TGN (DNA-thioguanine nucleotides) as a dose adjustment parameter as it represents the end cytotoxic metabolite of 6MP. In this review my objective is to evaluate what factors have potential as dose adjustment parameters during drug therapy for ALL. The publications reviewed in this review have been chosen according to set criteria and a thorough evaluation of search results on PubMed.

Keywords: *polymorphisms; Lymphoblastic Leukemia; Methotrexate; Thiopurine S-methyltransferase*

Introduction

Acute lymphoblastic leukemia (ALL) is a clonal acute malignant blood disease that most commonly occurs in the bone marrow through malignant transformation of B- or T-lymphocyte precursors. ALL is the most common cancer diagnosed in children and represents approximately 25% of cancer diagnosed among children younger than 15 years [1,2].

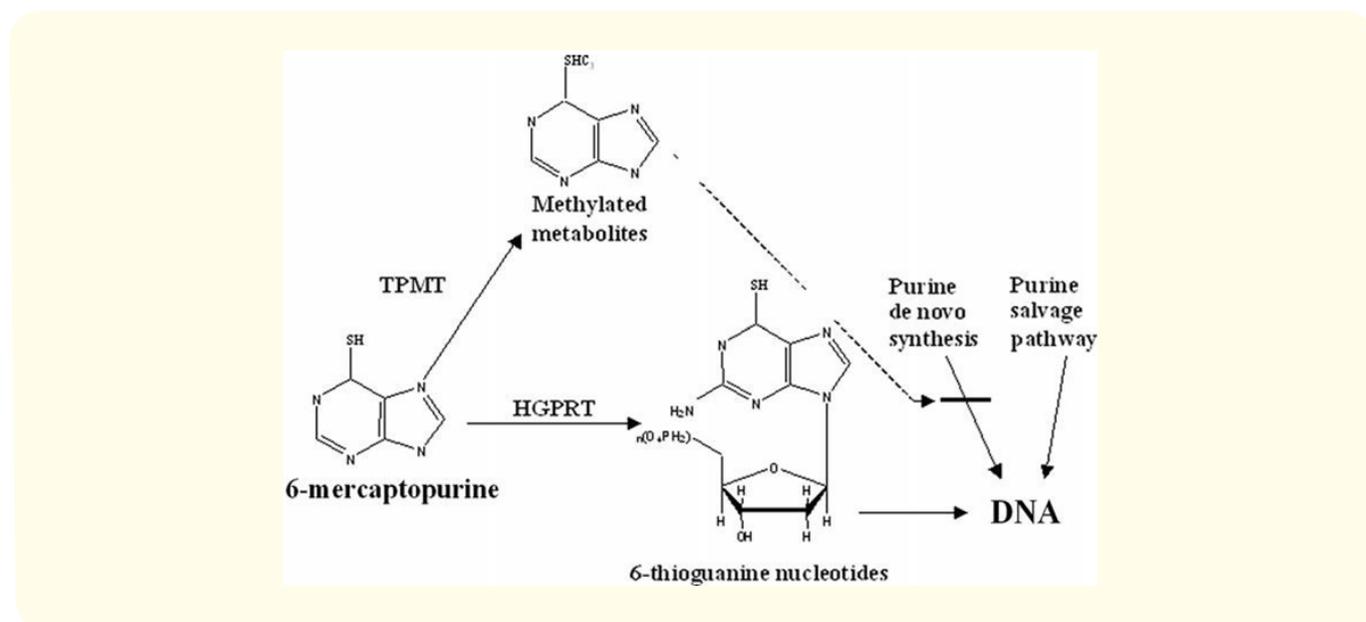
Due to improvements in a series of protocols where treatment has been risk-adapted the prognosis of these patients has been radically improved in the past decades [3]. Currently the cure rate is over 80% [4,5]. The current NOPHO ALL-2008 treatment protocol includes four main phases: induction, consolidation, delayed intensification and maintenance. The treatments total duration is two and a half years. Depending on the disease's character patients are assigned to a high, intermediate, or standard risk therapy [6-8]. In all the phases following induction, thiopurines (6-thioguanine or 6-mercaptopurine) are involved.

Thiopurines belong to a class of drugs that are called antimetabolites. Antimetabolites constitute a group of drugs that mask themselves as naturally occurring metabolites. This permits thiopurine incorporation into DNA and RNA. Thus this allows the antimetabolites to disturb normal cell function as they despite resemblance are not identical to the authentic DNA and RNA building blocks. Consequently

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antimetabolites interrupt normal cell division and development. As cancer cell's division is more extensive than that of normal cells an inhibition of this division constitutes a great injury to the tumor cells.

Furthermore thiopurines belong to a subgroup of antimetabolites called purine analogues. Purine is a normal building block in both RNA and DNA. Mercaptopurine is an example of purine analogues used while treating ALL. A central part of the ALL is treatment protocols is maintenance therapy with daily oral 6-mercaptopurine (6MP) and weekly Methotrexate (MTX) until 2-3 years from the time of diagnosis [9].

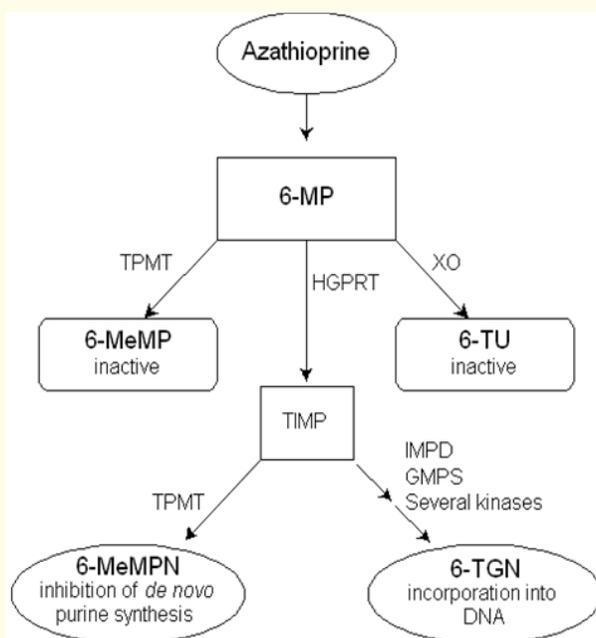


6-Mercaptopurine is a pro-drug which requires metabolic activation to obtain its cytotoxic effect. 6MP activity is chiefly caused by its transformation into thioguanine nucleotides (TGN) that are incorporated into the DNA of proliferating cells. Increasing levels of DNA-TGN will subsequently lead to apoptotic cell death through activation of the mismatch repair system [10-12]. Consequently DNA-TGN represents a 6MP endpoint metabolite and is accountable for its cytotoxic effect. Furthermore DNA-TGN is also responsible for 6MPs short and long term adverse events such as life-threatening myelosuppression and second malignant neoplasias [13].

TPMT is the enzyme that methylates and inactivates 6MP. However some of the 6MP methylated metabolites ex. Me TIMP efficiently inhibits de novo purine synthesis (Figure 1). As a result TGN incorporation increases due to diminished levels of competing endogenous purines [9,14]. However this mechanism does not compensate for the reductions in the TGN formation. TPMT variants are an important genetic cause of inter individual variability in the clinical response to 6MP. Thus in the NOPHO ALL-2008 protocol

The 6MP starting dose is regulated after the TPMT status [8,15-17]. High TPMT activity implicates a higher risk of treatment failure and relapses, while a low TPMT activity may involve higher cure rates as well as a higher risk of myelosuppression [17]. However TPMT polymorphism is not sufficient as an explanation for the variations in the clinical response. Thus other variants in other genes encoding 6MP metabolic enzymes are to be withheld in consideration [18-20].

As DNA-TGN represents the end-point 6MP metabolite it integrates the sum of the known and unknown upstream pharmacogenetic variability and therefore be used as a dose adjustment parameter.



Proper dosing of 6MP is still a major clinical challenge as the drug exhibits a low therapeutic index and substantial variations among patients. Standard dosing may result in fatal toxicities or under treatment in some patients [21-23]. A consistent dose-adjustment parameter for individual 6MP disposition is currently not available. The purpose of this review is to explore and evaluate what parameters can be implemented for dose-adjustment.

Study Objective

Currently the maintenance therapy for ALL according to the NOPHO ALL-2008 protocol involves a combination treatment with 6-mercaptopurine and methotrexate. As previously stated due to the variability in the patient drug metabolism suitable dosing regimens remain as a great clinical challenge. Thus individualized therapy is of great interest but has been inhibited by the possibilities to control the 6MP/ MTX metabolite levels in the system.

Measuring the accumulation of DNA-TGN is able to integrate the sum of the known and unknown upstream pharmacogenetic variability but has proven insufficient as DNA-TGN variations have failed to fully explain interindividual therapy variability. Thus the scope of factors with impact on the interindividual variability must come to embrace other relevant elements.

In this study my objective is to evaluate what factors have great potential as dose adjustment parameters during drug therapy for ALL. Hopefully this study will give new perspective on 6MPs pharmacokinetics and a broader range of potential dose adjustment parameters while developing possibilities for greater individualized chemotherapy.

Materials and Method

To find relevant articles the primary search database used was PubMed. While using the Mesh database peripheral results with minor relevance were excluded effectively. Furthermore the related articles search tab was also used to pinpoint other desired material. Articles were scanned according to their title's relevance to ALL and 6MP/MTX combinational therapy. Articles involving animals were excluded. Furthermore only articles in English or any of the Scandinavian languages were included. After a careful review of the resulting titles and abstracts articles were chosen. Reviews were excluded if not for the purpose of background information. 13 articles were selected on the basis of the eligibility criterias.

Search Term	Hits	Abstracts Read	Selected
("6-Mercaptopurine"[Mesh] AND Humans [Mesh]) AND (((("Methotrexate"[Mesh] AND Humans[Mesh])) AND (acute lymphoblastic leukemia AND Humans[Mesh])) AND (Humans[Mesh]))	471	20	4
((acute lymphoblastic leukemia AND Humans[Mesh])) AND ("6-Mercaptopurine/administration and dosage"[Mesh]) AND (Humans[Mesh])	399	39	10
("6-Mercaptopurine"[Mesh]) AND "Precursor Cell Lymphoblastic Leukemia-Lymphoma" [Mesh]	527	49	3

References in relevant articles were also used to find new articles. These articles are also included in the search result table above. Due to my affiliation to the Bonko Laboratory at Rigshospitalet as a research fellow I had extensive knowledge within the field prior to the commencement of this review. This also enabled me to have great insight into the newest trends within my field while my colleagues readily handed me their work within the field. Thus I had built an appreciation for the topic and many of the background references are part of comprehensive past searches. The full list of selected articles and a summary of their content is found in the appendix.

Results

Adequate dosage of thiopurines is vital while treating patients for ALL. Despite many advances in the treatment protocol for ALL over-dosage remains a serious issue as it may lead to increased risk of relapse and treatment interruptions. Studies have shown significant variations in the accumulation of thiopurine metabolites in red blood cells, that is associated with the possibility of toxicities and relapse. Yet this is not regarded as a superior pharmacological approach for dose-adjustment parameter than the established white blood cell counts (WBC) during therapy. According to the NOPHO ALL-2008 protocol, dose-adjustment in the maintenance phases is primarily guided by a target WBC of 1.5 - 3.0*10⁹/L. Thus current therapy is therefore guided by a target WBC to ensure efficacy. However it is known that all patients are not treated equally by this approach due to natural inter-individual variation in normal WBC-levels. The inter-individual variation is due to diversity in the intracellular metabolism and bioavailability. This has been studied with regard to single nucleotide polymorphisms (SNPs) in the enzyme thiopurine methyltransferase (TPMT), which S-methylates and (mostly) inactivates the thiopurine parent drugs and their nucleotides [24-27]. While myelosuppression is a possible fatal adverse event in thiopurine therapy it is essential to monitor WBC to avoid very low counts. Low WBC count may turn fatal due to opportunistic infection. As WBC levels during therapy also reflect the risk of relapse it provides as a valuable parameter for treatment intensity [13]. Since thiopurine metabolism is regulated by multiple enzymes, testing only TPMT genotype may not give sufficient information about the hematological toxicity.

ITPA is a phosphorylase that alters T1TP to T1MP (Figure 2). Studies have established an association between low-activity single nucleotide polymorphisms in ITPA with high levels of methylated 6MP metabolites, hepatotoxicity in the treatment of ALL

Tanaka, *et al.* and a potential greater risk of relapse [31]. Furthermore, Tanaka, *et al.* demonstrated that ITPA deficiency was a risk factor for leukopenia in patients receiving 6-MP therapy. The study by Dorababu *et al.* supports and acknowledges ITPA as an important contributor of inter-individual variations during ALL in treatment. Furthermore Dorababu, *et al.* [25] reported that epistatic interactions between the variations of *TPMT* (*3C, *12) and *ITPA* (rs1127354, rs8362) were associated with the 6-MP toxicity by multifactor dimensionality reduction analysis. Dorababu concludes that testing variants of *TPMT* and *ITPA* facilitates the pivotal individualization of the 6-MP therapy in children with ALL.

The antifolate methotrexate (MTX) is used as an antineoplastic agent in the treatment of ALL together with 6-MP. Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism. It catalyzes the conversion of 5, 10 methylene tetrahydrofolate to 5-methyltetrahydrofolate in the folic acid cycle. The study conducted by Liu, *et al.* showed that

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Furthermore individuals with *TPMT* gene variants associated to decreased enzymatic activity are at greater risk of adverse effects while treated according to standard protocols. However these patients do not experience greater risk of adverse effects in comparison with patients with wild-type *TPMT* if their mercaptopurine dose-adjustment is aligned according to their *TPMT* status. *TPMT* genotyping before the initiation of 6MP therapy is thus a cost-effective strategy to individualize thiopurine dosing, lower incidence of adverse effects and increase the efficacy of the treatment. However it is simply not only the *TPMT* gene that determines how each individual responds to 6MP/MTX treatment. The inter-individual differences in treatment response cannot be fully explained by *TPMT* gene variants.

Additional genes must be involved in the determination of adverse events to multi-agent chemotherapy. *ITPA* is also regarded as a great determinant of mercaptopurine metabolism and the risk of toxicity. This greatly demonstrates the development of pharmacogenetics in the clinic; as treatment is individually tailored according to one genetic determinant of drug response, the significance of other genetic polymorphisms appear. While there is a general concordance that homozygous and heterozygous polymorphisms of *TPMT* may cause toxicity and relapse in children with ALL, no other polymorphisms have the same indisputable association with outcomes among the studies reviewed. The reasons for inconsistent results among studies may include treatment protocol, sample size, laboratory methods, and specimen quality and data analysis.

Analyzing genes directly or indirectly involved in the folate metabolism (ex. *MTHFR*) can add valuable additional information to the standard *TPMT* genotyping and thus enable the development of improved efficiency and safety of the thiopurine therapy. The study conducted by Shimasaki et al showed how certain polymorphisms in the *MTHFR* gene were associated with interruptions in both 6MP and the MTX dosing. Thus *MTHFR* polymorphism may be a promising candidate for further investigation in future pharmacogenomic studies.

However even though gene variants clarify a great part of the inconsistency and pharmacogenetically individualized therapy has improved results (e.g. adjustment of 6MP dosage according to *TPMT* status), it remains insufficient as an explanation for variations in clinical response.

One must bear in mind that pharmacogenetic studies are limited to polymorphisms in enzymes that are already determined as significant. However in patients undergoing treatment for ALL many proteins are involved in intracellular transportation, metabolism, drug absorption etc. These proteins may very well play a fundamental role with high impact on the inter-individual variations. Furthermore ALL treatment might involve other drugs than the standard 6MP/MTX therapy such as supportive care and over-the-counter medications. These variables might be neglected or quantified with difficulty during studies of 6MP/MTX metabolism. Also the studies presented have important limitations that are frequently present in pharmacogenetic studies. Many of these studies are retrospective with modest sample sizes, varied inclusion criteria and inconsistent outcomes. Moreover, most drug responses are influenced by multiple genes, thus polygenetic studies and models are essential while appreciating the full scope of the genetic determinants of drug response. It is a key to evaluate and analyze non-genetic factors ex. patients' and physicians' compliance as they to play a role in treatment procedure.

Hence the future of individualized therapy does not solely rely on the ability to identify and evaluate new genetic determinants of ALL therapy. The search for determining factors must appreciate other areas and not merely focus on the pharmacogenomic aspect. Therefore another challenge clinicians face while developing optimal treatment approaches is how to form a conjunction between obtained genetic information and data on nongenetic causes of interpatient variability in drug response.

DNA-TGN

Emerging studies have investigated quantification of DNA-TGN as a dose adjustment parameter as it represents the end cytotoxic metabolite of 6MP. Monitoring and adjusting ALL therapy by DNA-TGN quantification may lead to a lower risk of relapse and toxicity. Investigating DNA-TGN distribution in cells is crucial while gaining insight into the causes behind inter-individual variability during treatment response.

Gender

Some studies have elucidated sex as a factor well worth investigating as a determinant during ALL therapy. In some ALL treatment reports it has been noted that boys have tolerated 6-MP better than girls as they were prescribed higher doses and experienced fewer dose reductions due to myelosuppression [32]. Jacobsen's study also displayed differences in DNA-TG between boys and girls which reflect gender-based differences in disposition towards thiopurines. Thus future studies might want to delve further into the gender based differences in treatment.

Ethnicity

Studies on certain ethnic groups prove that certain genetic patterns are more common among them and thus potentiate certain drug metabolic patterns [31]. For instance the Kim et al study suggests that there are other possible pharmacogenetic factors besides *TPMT* or *ITPA* polymorphisms which influence the metabolism of mercaptopurine in Asian populations. Thus the future of individualized therapy must consider ethnicity as a great source of genetic polymorphisms. It is not sufficient to carry out studies on polymorphisms on Caucasian populations. For instance according to the institutional experience (Asia) while treating patients according to the Western ALL protocols, numerous patients could not endure the full doses [31].

In conclusion it is of great importance to study and analyze the different distributions of genetic polymorphism among different ethnic populations as most previous pharmacogenetic studies have predominantly been conducted on Western populations.

Mitochondrial DNA

While conducting this review it has been evident that the research focus has primarily been directed at the nuclear DNA as the primary contributor while studying pharmacogenetics. Thus another important organelle with genetic content has not been properly addressed; the mitochondria. It is well established that the mitochondria is important in the different aspects of our health. Their unique oxidative inner milieu with unprotected chromosomes suggests that they are particularly vulnerable and exposed to DNA [33]. Furthermore the mitochondria have a central role in the folate metabolism which constitutes the target of the methotrexates. This suggests a biochemical deviation that has direct impact on the 6MP/MTX combinational therapy. Thus the mitochondria should not be neglected while studying the pharmacogenetic basis for inter-individual variations during 6MP/MTX therapy.

A study conducted by Kwok CS., *et al.* [34] showed that certain polymorphisms among ALL patients mtDNA were frequently associated with good response to chemotherapy [34]. Furthermore Kwok., *et al.* found an association between specific polymorphisms with prognostically important subgroups of leukemia. It appears that polymorphisms at and around certain areas of transcriptional control may potentially influence response to chemotherapy. According to Kwok., *et al.* [34] mtDNA content and polymorphisms around the origin of O_H and the binding site for mitochondrial transcription factor may propose an association between mtDNA replication and pathogenesis of childhood leukemia. Studies have also reported that the decrease of mtDNA in childhood ALL samples after treatment may contribute to the improved prognosis of childhood ALL.

Conclusion

Individualizing drug therapy through a pharmacogenomic approach presents an opportunity to enhance drug efficacy, reduce the risk of adverse effects and is a cost-effective treatment strategy. Despite the identification of many pharmacogenetic markers, pharmacogenetic testing has been implemented in clinical practice for only a small number of drugs. Furthermore the reviewed publications exemplified and profoundly highlighted that the metabolism of a given drug is not based solely on a single drug-metabolizing enzyme, but involves a complex enzyme network of competing metabolic pathways.

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It has become apparent that the identification of relevant pharmacogenetic markers is much more complex than initially believed. Thus the pharmacogenetic approach has its weaknesses. Another difficulty is compliance within the clinical staff. Compliance may pose as a problem while clinicians neglect to adjust dosage according to pharmacogenomic testing. Many times clinicians disregard to implement potentially valuable genotype and phenotype data into their practice as dose adjustments may lead to increased risk of adverse effects. It is thus important to educate clinicians to implement outcome predictors in their routine clinical decision-making.

Author	Study Design	Factor Evaluated	Reviewer Conclusion
Wennerstrand, et al. [24]	Prospective case study	Thiopurinemethyltransferase(TPMT)enzymeactivity	Results indicate that TPMT genotyping should be performed in children with ALL
Dorababu P, et al. [25]	Prospective case study	Thiopurinemethyltransferase(TPMT)and inosinetriphosphatepyrophosphatase(ITPA)	Testing variants of TPMT and ITPA facilitates tailoring of the 6-MP therapy in children with ALL
Liu SG, et al.	Retrospective study	Methylenetetrahydrofolate reductasegenepolymorphisms	Genotyping of MTHFR and measurement of plasma MTX levels might be useful to optimize MTX therapy.
Peregud-Pogorzelski J, et al. [26]	Case-control study	Thiopurine S-methyl transferase (TPMT) polymorphisms	Results indicate TPMT's genotype influence on the safety and efficacy of ALL treatment.
LundB	Retrospective study	Several	Treatment related deaths (TRD) were potentially preventable and several factors were recognized to increase TRDs prevalence.
Hedeland RL, et al. [9]	Retrospective case study	6-thioguaninenucleotide levels (DNA-6TGN)	DNA-6TGN as a relevant pharmacokinetic parameter for monitoring 6MP treatment intensity compared to erythrocyte 6MP metabolites levels.
Shimasaki N, et al.	Retrospective study	Methylene tetrahydro folate reductase (MTHFR)677C/Tor reduced folate carrier1(RFC1)80G/A polymorphisms	Study does not prove but suggests thatMTHFR677C/T and RFC1 80G/A polymorphisms may serve as predictors of toxicity during maintenance chemotherapy.
Andersen JB, et al. [27]	Case study	Thiopurine methyltransferase (TPMT) enzyme activity	Results indicate that TPMT genotyping should be performed in children with ALL
Schmiegelow K (1994) [13]	Prospective case study	Erythrocyte concentration of MTX polyglutamates and 6-thioguanine nucleotides (E-MTX and E-6TGN, the respective major cytotoxic metabolites of MTXand6MP) and WBC	Results indicate that E-MTX and E-6TGNmaygive a better reflection of the treatment intensity than do the WBCs alone.
Jack Hummel and Jacobsen	PhD thesis; several	DNA-TG	DNA-TG during the maintenance therapy for ALL could potentially lead to improvement in outcomes
Ebbesen MS	Case-control study	DNA-TG	DNA-6TGN as a relevant pharmacokinetic parameter for monitoring 6MP treatment intensity
Tanaka, et al.	Retrospective case study	Inosinetriphosphate pyrophosphatase (ITPA)	Measuring ITPA activity is important for ensuring the safety of maintenance therapy for Asians with ALL because thiopurine S-methyl transferase mutations are rare in the Asian population
Kim, et al. [31]	Retrospective case study	Thiopurinemethyltransferase (TPMT) and inosinetriphosphatepyrophosphatase (ITPA) etc.	Other possible pharmacogenetic factors besides TPMT or ITPA polymorphisms which influence the metabolism of mercaptopurine in Asian populations

Furthermore it is now more widely recognized that the adverse effects of 6-MP/MTX therapy are under polygenic rather than monogenic control. Thus this must be implemented with greater conviction while studying the pharmacogenomics of ALL treatment. Recognizing the genetic variations with impact on 6-MP pharmacokinetics and response has potential to enhance risk stratification and event-free survival rates in children with ALL.

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